Risk Factors for Maternal Chagas Disease and Vertical Transmission to Infants in a Bolivian Hospital

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Abstract

Introduction: Vertical transmission of *Trypanosoma cruzi* infection accounts for a growing proportion of new cases of Chagas disease. Congenital Chagas disease is curable if treated promptly, but the majority of infected infants do not receive timely diagnosis or treatment. Better risk stratification is needed to predict which women are more likely to have and transmit the infection.

Methods: This study enrolled women and their infants at the Percy Boland Women's Hospital in Santa Cruz, Bolivia. Pregnant women were screened for *T. cruzi* by serological rapid test. Infants of seropositive mothers underwent diagnostic testing with microscopy ("micromethod") and quantitative polymerase chain reaction (qPCR) as newborns and at one- and nine-month follow-up.

Results: Among 5,828 enrolled women, 1,271 (21.8%) were seropositive for Chagas disease. Of the 1,325 total infants of seropositive mothers, 113 infants (8.5%) were diagnosed with congenital Chagas disease by microscopy or qPCR. In a multivariate logistic regression, older maternal age, family history of Chagas disease, lack of maternal high school education, and multiple environmental factors were significantly associated with higher odds of maternal Chagas disease. Cesarean delivery was found to be protective against vertical transmission, adjusted for twin delivery (adjusted OR: 0.63, 95% CI: 0.41-0.98, p=0.040). Among infants born to mothers with Chagas disease, congenital infection was more common in twins (adjusted OR:

3.30, 95% CI: 1.97-5.54, p<0.001) and male infants (adjusted OR: 1.50, 95% CI: 1.01-1.22, p=0.045).

Discussion: A better understanding of risk factors for maternal and congenital Chagas disease may help improve regional initiatives to reduce disease burden.

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Introduction:

Almost 6 million people worldwide are infected with *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease.[1] A neglected tropical disease, Chagas disease is endemic to 21 countries in Latin America and is a major source of morbidity and mortality in this region.[2] Bolivia has the highest prevalence of Chagas disease in the world, with over 6% of the overall population estimated to be infected with *T. cruzi*.[1] In a 2013 analysis, Chagas disease was associated with an annual global burden of over 800,000 disability-adjusted life years (DALYs) and $600 million (USD) in health care costs.[3] Chagas disease continues to grow as a global public health issue due to widespread migration.

The majority of new cases with *T. cruzi* occur through vector-borne transmission by the triatomine bug (also known as the "kissing" bug).[4,5] When the triatomine bug bites a human, feces infected with *T. cruzi* inoculates the skin or mucosa. Transmission may also occur through vertical transmission from mother to infant, blood transfusion, or consumption of contaminated food.[6] Since the 1990's, regional initiatives for the prevention of Chagas disease have significantly reduced vector-borne and blood-borne transmission rates.[7] As a result, congenital infection has become an increasingly important source of transmission and now accounts for 22% of new cases.[8]

Vertical transmission occurs in approximately 5% of chronically infected mothers.[8] Transmission rates vary widely between studies but appear to be higher in endemic settings, which may be related to higher parasitemia, different strains of *T. cruzi*, or falsely inflated rates due to vector transmission in infants. Multigenerational transmission of Chagas disease has also

been reported, as congenitally infected females eventually pass the infection to their own infants.[9–11] A small subset of congenitally infected infants presents with severe disease characterized by respiratory insufficiency, meningoencephalitis, hepatosplenomegaly, or anemia.[8,12] However, the majority of infants are asymptomatic or have nonspecific symptoms, and thus rely on appropriate testing for diagnosis.[12] Regardless of initial presentation, infants with congenital infection are presumed to have up to a 30% risk of potentially fatal cardiomyopathy or gastrointestinal disease later in life.

Potentially curative treatment exists for acute *T. cruzi* infection, including therapy with benznidazole or nifurtimox. Because treatment is most effective and best tolerated in the first months of life, accurate screening in high-risk newborns is critical.[13] In most endemic countries, the diagnostic algorithm for congenital infection relies on serological screening in the mother and microscopy, or "micromethod," in the infant. Micromethod, which involves examination of peripheral blood for parasites, detects less than half of congenital infections.[14–16] More sensitive diagnostics exist, such as quantitative polymerase chain reaction (qPCR), but these techniques are often unavailable in endemic settings due to the need for expensive imported equipment and technician expertise.[13] Consequently, the majority of infants with congenital Chagas disease do not receive timely diagnosis or treatment in many endemic settings.[16,17]

Given the low rates of diagnosis and treatment of congenital Chagas disease despite the availability of treatment, better risk stratification is needed to predict which women are more likely to transmit the infection. Currently, our understanding of the risk factors for vertical transmission of *T. cruzi* infection is limited. Various maternal, epidemiological, and parasitic factors have been implicated in risk of transmission, but studies have often produced conflicting results.[7,16,18]

This study aims to describe the epidemiology of congenital Chagas disease in an endemic setting at the Percy Boland Women's Hospital in Santa Cruz, Bolivia. The largest city in Bolivia, Santa Cruz has an estimated *T. cruzi* prevalence of 20-30% among pregnant women.[16,17] This study also aims to identify demographic, medical, socioeconomic, and environmental risk factors for maternal Chagas disease and vertical transmission of *T. cruzi* infection.

Materials and Methods:

Setting and participants:

The study took place at Percy Boland Women's Hospital in Santa Cruz, Bolivia, which delivers approximately 8,000 infants each year. Mothers were eligible if they presented for delivery at the Percy Boland Women's Hospital and gave informed consent. Mothers under the age of 18 were allowed to participate if they were emancipated minors, defined as married or living independently from their parents. Mothers gave informed consent for themselves and their newborns. The consent process was conducted by local investigators and trained study nurses.

Maternal and infant data:

Study personnel conducted a brief survey with participating mothers about medical history, obstetric history, and sociodemographic characteristics. Sociodemographic data included age, place of birth, address, highest attained education level, and profession. Data on the participants' most recent delivery were obtained from the written patient medical record. Survey responses were recorded by study personnel using secure pre-programmed cell phones.

Participating women received perinatal screening for Chagas disease through a finger-prick rapid test (InBios Chagas DetectTM Plus; reported sensitivity: 96.2%, reported specificity: 98.8%).[19] Women with a positive rapid test underwent confirmatory serologic testing with indirect hemagglutination (IHA, Wiener lab IHA Chagatest) or enzyme-linked immunosorbent assays (ELISA) with recombinant antigens (Wiener lab BioELISA Chagatest). In accordance with World Health Organization (WHO) guidelines, positive results from two separate immunoassays were required for a diagnosis of Chagas disease.[19]

Infants of mothers with a positive Chagas disease rapid test were enrolled in the cohort (Figure 1). Testing included perinatal microscopic examination for *T. cruzi* organisms in peripheral blood ("micromethod") and qPCR performed perinatally and at one- and nine-month follow-up. Among infants whose mother was seropositive for *T. cruzi* infection with confirmatory serology, a positive micromethod or qPCR within the first year of life was considered diagnostic for congenital Chagas disease.

All maternal serological testing and neonatal micromethod were performed onsite in the maternity hospital's laboratory. DNA extraction from blood samples and qPCR testing for *T. cruzi* were performed at the Infectious Diseases Research Laboratory of the Universidad Peruana Cayetano Heredia in Lima, Peru. Duplex real-time qPCR was performed targeting the *T. cruzi* nuclear genome satellite sequence and a recombinant plasmid (pZErO-2) as internal amplification control (IAC), according to published methods.[20] Cycling conditions were 10 minutes at 95°C, amplification at 95°C for 15 seconds and 58°C for one minute for 40 cycles, and extension at 72°C for one minute followed by a 4°C hold.

Mothers whose infants were diagnosed with congenital Chagas disease by either micromethod or qPCR were contacted by study personnel and referred for treatment of the infant with benznidazole, according to the national guidelines of the Chagas Program in Bolivia.

This protocol was approved by the ethics committee of the Bolivian Catholic University, international registration FWA 0017928 and PRISMA 00001219. Study analysis was approved by the IRB at the University of North Carolina at Chapel Hill (IRB 19-3014).

Statistical analysis:

Data were analyzed using Stata (version 16). We calculated descriptive statistics of the overall population and stratified by maternal rapid test result. We performed univariate and multivariate logistic regressions to examine predictors of maternal infection with *T. cruzi*. We considered 22 variables as potential covariates (Table 2). We excluded previous diagnosis and treatment of Chagas disease from the final model due to potential collinearity. We set an a priori cutoff of 5% missing data to exclude other variables from the final model. We also set an a priori alpha criterion of 0.05 to remove variables that were not statistically contributing to the model and were thus subsequently removed from the model. Collinear variables (VIF approaching 10) were removed from the final model. We used the likelihood ratio test to determine if the reduced model was significantly different from the fully adjusted model using an alpha criterion of 0.05.

We subsequently performed univariate and multivariate logistic regressions to examine maternal predictors of congenital infection with *T. cruzi* (Table 3). Only infants of mothers with confirmed serology for Chagas disease were included in this analysis. We considered the same variables as above, as well as having a Cesarean delivery, premature rupture of membranes (PROM), or twin birth. We set an a priori alpha criterion of 0.05 to remove variables that were

not statistically contributing to the model and were thus subsequently removed from the model. We used the likelihood ratio test to determine if the reduced model was significantly different from the fully adjusted model using an alpha criterion of 0.05.

We also separately examined two infant risk factors for congenital Chagas disease, being a twin and sex (Table 4). We performed individual univariate logistic regressions and a combined multivariate logistic regression, using a priori alpha criterion of 0.05 for statistical significance.

Results:

From May 2016 to 2019, 5,828 pregnant women were enrolled (Figure 1). A total of 5,643 singleton births, 181 twin births, and 4 triplet births resulted in a total of 6,017 infants delivered, including live births and stillbirths.

The average age of enrolled women was 27.6 years old (SD: 7.3). Among enrolled women, 1,287 had a positive Chagas rapid test. Of these, 1,271 (98.8% of women with a positive rapid test; 22.0% of all enrolled women) had positive confirmatory serology with IHA or ELISA.

Of the 1,325 infants whose mothers were seropositive for Chagas disease, 113 total infants (8.5% of infants with seropositive mothers, 1.9% overall) were diagnosed with congenital infection by micromethod or qPCR (Table 1). Of these, 87 infants (77.0%) were diagnosed by micromethod as newborns. An additional 26 infants (23.0%) with negative micromethod were subsequently diagnosed with qPCR during newborn or one-month testing. Of the 1,325 infants with seropositive mothers, only 272 (20.5%) returned for nine-month follow-up with qPCR testing. No new diagnoses were made through qPCR results at nine months.

Risk Factors for Maternal Infection:

A total of 20 risk factors were found to be significantly predictive of maternal infection with *T. cruzi* in the univariate analysis (Table 2). Demographic factors associated with higher odds of maternal infection included older maternal age and parity. Medical factors associated with higher odds of maternal infection included previous diagnosis of Chagas disease, previous treatment for Chagas disease, family history of Chagas disease, history of receiving donated blood, diagnosis of diabetes mellitus, positive maternal VDRL, and positive maternal toxoplasmosis. Socioeconomic factors associated with lower odds of maternal infection included maternal high school education (versus no high school education), paternal high school education, maternal career as a technician or professional (versus a career as a homemaker, laborer, service industry worker, or other), paternal career as a technician or professional, number of bedrooms in the home, and number of household amenities. Environmental factors associated with higher odds of maternal infection included having seen triatomine bugs in the home, having ever lived in a home with mud walls, having ever lived in a home with a roof made of palm, straw or hollow reeds, having ever lived in a home with a dirt floor, or having ever lived in a rural area. The number of people living in the home and number of rooms in the home were not significantly associated with odds of maternal infection (p=0.955 and p=0.073, respectively).

A total of seven risk factors were found to be significantly predictive of maternal infection with *T. cruzi* in the final multivariate analysis (Table 2). Risk factors associated with higher odds of maternal infection included older maternal age (adjusted OR: 1.04, 95% CI: 1.03-1.05, p<0.001), positive family history of Chagas disease (adjusted OR: 3.18, 95% CI: 2.75-3.67, p<0.001), having seen triatomine bugs in the home (adjusted OR: 3.49, 95% CI: 2.88-4.23, p<0.001), having ever lived in a home with mud walls (adjusted OR: 1.64, 95% CI: 1.26-2.12,

p<0.001), and having ever lived in a home with a dirt floor (adjusted OR: 1.83, 95% CI: 1.42-2.36, p<0.001). Protective factors associated with lower odds of maternal infection included maternal high school education (adjusted OR: 0.65, 95% CI: 0.55-0.75, p<0.001) and having ever lived in a rural area (adjusted OR: 0.70, 95% CI: 0.58-0.84, p<0.001). Combined, these risk factors accounted for 20.9% of the variance in maternal infection status.

VDRL and toxoplasmosis results were excluded from the final model because they exceeded our a priori limit of 5% missing data. Parity and number of home amenities were removed from the model due to collinearity. We determined that the other variables were not significant covariates due to an alpha criterion greater than 0.05; we confirmed that our final, reduced model was not significantly different than our fully adjusted model using the likelihood-ratio test.

Maternal Risk Factors for Vertical Transmission:

Among seropositive mothers, two risk factors were found to be independently, significantly predictive of vertical transmission of *T. cruzi* infection in the univariate analysis: previous maternal diagnosis of Chagas disease and having a twin birth (Table 3).

Two risk factors were found to be significantly predictive of vertical transmission in seropositive mothers in the final multivariate analysis. Having a Cesarean section rather than other forms of delivery was found to be a protective factor (adjusted OR: 0.63, 95% CI: 0.41-0.98, p=0.040). Having twins rather than a singleton birth was a significant risk factor for vertical transmission (adjusted OR: 3.17, 95% CI: 1.53-6.55, p=0.002). Combined, these factors accounted for 1.6% of the total variance in vertical transmission.

Previous maternal diagnosis of Chagas disease was not adapted into a multivariate model due to potential collinearity. We determined that the other variables were not significant covariates due to an alpha criterion greater than 0.05.

Infant Risk Factors for Congenital Infection:

Among infants born to seropositive mothers, being a twin was independently, significantly associated with higher odds of vertical transmission of *T. cruzi* in the univariate analysis (OR: 3.25, 95% CI: 1.94-5.44, p<0.001) (Table 4). In the multivariate analysis, being a twin (adjusted OR: 3.30, 95% CI: 1.97-5.54, p<0.001) and male (adjusted OR: 1.50, 95% CI: 1.01-2.22, p=0.045) were associated with higher odds of congenital Chagas disease (Table 3). Combined, these risk factors accounted for 2.7% of the total variance in infant infection status.

Discussion:

At a maternity hospital in Santa Cruz, Bolivia, our study yielded a *T. cruzi* seroprevalence of 21.8% among pregnant women and a vertical transmission rate of 8.5%. This seroprevalence is somewhat lower than in the most recent Chagas cohort study in Santa Cruz, which demonstrated a 26% prevalence from 2010-2014.[21] This difference could be explained by gradual community-wide reductions in Chagas disease due to regional vector control initiatives, as well differences between hospital populations or maternal rapid test techniques. The vertical transmission rate in our study is similar to the previous study, which demonstrated a 7.8% transmission rate.

Our study identified several risk factors for maternal Chagas disease, which combined accounted for approximately 20% of variation in disease status. Older maternal age was

independently associated with higher odds of maternal infection. This is consistent with findings from past studies and may be related to housing improvements and vector control initiatives, which have reduced seroprevalence of *T. cruzi* infection over time in many communities.[22–27] Family history of Chagas disease was also independently associated with higher odds of maternal infection, which may reflect environmental risk factors or multigenerational vertical transmission. Notably, pregnant women with a high school education had significantly lower odds of Chagas disease than women without a high school education. This result confirms the findings of several previous studies and highlights the importance of socioeconomic inequalities in maternal risk for Chagas disease.[22,24,25]

In the univariate analysis, maternal history of blood transfusion, diabetes mellitus, positive VDRL, and positive toxoplasmosis were associated with higher odds of infection. However, blood transfusion and diabetes mellitus were not significantly associated with maternal infection when adjusted for other variables in the multivariate analysis. VDRL and toxoplasmosis status were not included in the multivariate model due to the high percentage of missing data. Future studies may wish to examine whether VDRL and toxoplasmosis are independent risk factors for maternal infection.

Housing materials are an established risk factor for Chagas disease, as triatomine vectors thrive in houses with mud walls and thatched roofs.[28] Our study found that history of living in a home with mud walls or dirt floor or having seen triatomine bugs in the home were independently associated with higher odds of maternal infection. Interestingly, although history of living in a rural area was associated with higher odds of maternal infection in the univariate analysis, it was a protective factor in the multivariate analysis. Although rural dwelling has previously been shown to be a risk factor for infection, it may be a proxy for poor housing

construction, lower educational levels, or triatomine infestations. Rural dwelling may not be an independent risk factor for infection when adjusted for these factors. The reasons for rural dwelling being protective against infection are unclear but could be related to immune tolerance or increased awareness of vector transmission. This would be consistent with findings that prolonged or high levels of vector exposure, such as living in a triatomine-infested house, are associated with reduced risk of vertical transmission.[29–32]

Our study also identified maternal and infant risk factors for vertical transmission, which combined accounted for a small amount of variance in infant disease status. Notably, Cesarean section was associated with lower odds of vertical transmission when controlled for twin births. To our knowledge, this is the first study investigating the association of birth type with risk of vertical transmission of *T. cruzi*. Because the multivariate analysis only included delivery type and twin birth, it is unclear whether the association with Cesarean section could be related to socioeconomic factors. This question warrants additional research, as Cesarean section has been shown to be protective against vertical transmission of certain infectious diseases, such as human immunodeficiency virus (HIV).[33] Twins and male infants also had significantly higher odds of congenital Chagas disease. The association between twins and risk of vertical transmission has demonstrated in two previous studies and may be related to placental insufficiency or enhanced immune suppression.[29,34] The association between infant sex and risk of transmission has only been evaluated in a handful of studies, with conflicting results.[21,32,35] Future studies are needed to examine whether sex-based hormonal or genetic differences contribute to susceptibility to vertical transmission.

Our study has several limitations. First, diagnosis of congenital Chagas disease relied primarily on perinatal and one-month microscopy and qPCR, as the majority of infants did not

complete nine-month follow-up (Figure 1). In addition, due to loss to follow-up and laboratory closures due to COVID-19, we did not have access to nine-month serology results, which are considered the gold standard for congenital Chagas disease diagnosis.[21] As a result, many cases of vertical transmission were likely missed. Unfortunately, low follow-up rates are common in congenital Chagas disease cohort studies in endemic, low-resource setting.[16,21,36] Barriers to follow-up include perceived low risk to infants and the cost of travel to the hospital, particularly for families living in remote, impoverished areas. Our study is also limited by its reliance on self-reported data for environmental exposures and medical history, which could introduce recall bias.

Conclusions:

Overall, our cohort study in a high-burden, low-resource setting in Santa Cruz, Bolivia adds to a growing body of literature of risk factors for maternal Chagas disease and vertical transmission to infants. Our findings suggest that older age, family history of Chagas disease, educational level, and environmental exposures contribute to maternal risk of Chagas disease. It also suggests that Cesarean delivery may be protective against vertical transmission, while twins and male infants may have increased risk of infection. Future studies are needed to validate these findings and consider additional predictors of vertical transmission, as the risk factors in our study contributed to a small proportion of overall variance. A better understanding of risk factors for vertical transmission of *T. cruzi* infection may improve risk stratification in high-risk populations and reduce disease burden.

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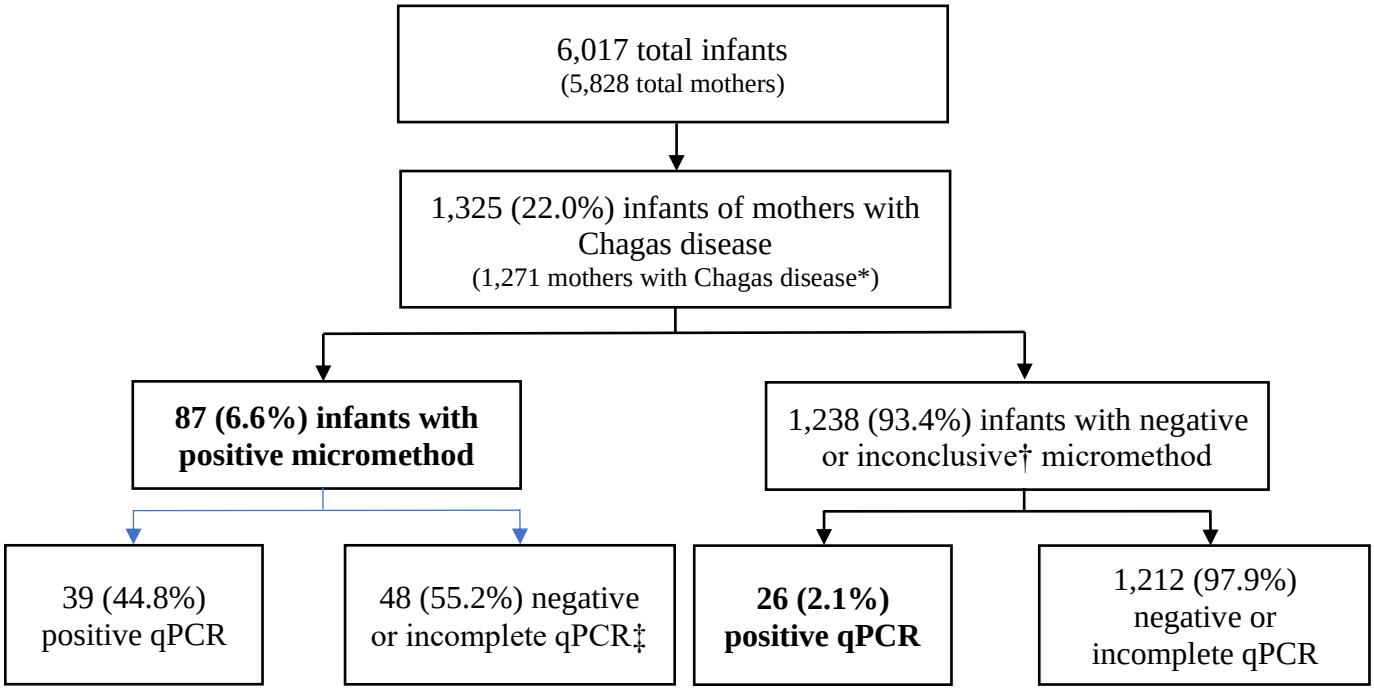
Figure 1. Overview of diagnostic testing for congenital *T. cruzi* infection.



* Maternal Chagas disease was diagnosed by positive rapid test followed by confirmatory testing with IHA or ELISA

† 3 infants whose mother was serologically positive for *T. cruzi* received micromethod testing but had inconclusive results due to laboratory error. None of these infants had a subsequent positive qPCR test result.

‡ Of these infants, 16 had at least one negative qPCR during follow-up. The remainder (n=32) did not complete qPCR testing as a newborn and did not return for follow-up.

Table 1. Summary of diagnostic results among infants of seropositive mothers

	Micromethod positive (n)	Micromethod negative (n)	Total
qPCR positive (n)	39	26	65
qPCR negative or incomplete* (n)	48	1,212	1,260
Total	87	1,238	1,325

Table 2. Maternal demographics and risk factors for chronic Chagas disease

	Women with Chagas disease (n=1,271) Mean ± SD or n (%)	Women without Chagas disease (n=4,557) Mean ± SD or n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Demographics				
Age	27.6 ± 7.3	24.5 ± 6.9	**1.06 (1.05-1.07)**	**1.04 (1.03-1.05)**
Parity	3.3 ± 1.8	2.7 ± 1.7	**1.19 (1.15-1.23)**	-
Medical history				
Previous diagnosis of Chagas disease	780 (61.4%)	171 (3.8%)	**40.7 (33.7-49.3)**	-*
Previous treatment for Chagas disease	38 (3.0%)	11 (0.2%)	**12.7 (6.5-25.0)**	-*
Family history of Chagas disease	746 (58.7%)	1264 (27.7%)	**3.70 (3.25-4.21)**	**3.18 (2.75-3.67)**
History of receiving donated blood	196 (15.4%)	502 (11.0%)	**1.47 (1.23-1.76)**	-
Diabetes mellitus	18 (1.4%)	36 (0.8%)	**1.80 (1.02-3.19)**	-
VDRL†			**3.34 (1.47-7.60)**	-
Positive (reference)	11 (1.5%)	12 (0.4%)		
Negative	736 (98.5%)	2682 (99.6%)		
Not done/unknown	524	1863		
Toxoplasmosis †			**2.19 (1.15-4.15)**	-
Positive (reference)	25 (49.0%)	51 (30.5%)		
Negative	26 (51.0%)	116 (69.5%)		
Not done/unknown	1220	4390		
Socioeconomic factors				
Maternal high school education			**0.64 (0.56-0.73)**	**0.65 (0.55-0.75)**
Yes (reference)	396 (31.2%)	1880 (41.3%)		
No	873 (68.8%)	2667 (58.7%)		
Unknown	2	10		
Paternal high school education			**0.68 (0.60-0.77)**	-
Yes (reference)	503 (39.6%)	2233 (49.1%)		
No	766 (60.4%)	2313 (50.9%)		
Unknown	2	11		
Maternal career			**0.69 (0.52-0.91)**	-
Professional or technician (reference)	66 (6.2%)	319 (8.8%)		
Other ‡	996 (93.8%)	3312 (91.2%)		
Unknown	209	926		
Paternal career				-

Professional or technician (reference) Other ‡ Unknown	433 (39.2%) 672 (60.8%) 166	1686 (43.4%) 2203 (56.6%) 668	**0.84 (0.73-0.96)**	
Number of people living in home	5.7 ± 2.6	5.7 ± 2.9	1.00 (0.98-1.02)	-
Number of bedrooms in home	2.3 ± 1.4	2.4 ± 1.5	**0.95 (0.91-0.99)**	-
Number of total rooms in home	4.3 ± 1.8	4.4 ± 1.9	0.97 (0.94-1.00)	-
Number of household amenities	3.0 ± 0.9	3.1 ± 0.9	**0.86 (0.80-0.92)**	-
Environmental factors				
Having seen triatomine bugs in the home	740 (58.2%)	780 (17.1%)	**6.75 (5.89-7.73)**	**3.49 (2.88-4.23)**
Having lived in a home with mud walls	714 (56.2%)	859 (18.9%)	**5.52 (4.83-6.31)**	**1.64 (1.26-2.12)**
Having lived in a home with a roof made of palm, straw, or hollow reeds	530 (41.7%)	594 (13.0%)	**4.78 (4.14-5.49)**	-
Having lived in a home with a dirt floor	673 (53.0%)	772 (16.9%)	**5.52 (4.82-6.31)**	**1.83 (1.42-2.36)**
Having lived in a rural area	834 (65.6%)	2009 (44.1%)	**2.42 (2.13-2.76)**	**0.70 (0.58-0.84)**

Variables not significantly contributing to the multivariate model were removed from the final reduced model (see Methods). Home amenities included electricity, television, refrigerator, personal form of transportation, and computer.
In the final multivariate model, odds ratios were adjusted for all other variables found to significantly contribute to the model: age, family history of Chagas disease, maternal high school education, having seen triatomine bugs in the home, having lived in a home with mud walls, having lived in a home with a dirt floor, and having lived in a rural area.
*Excluded from multivariate model (see Methods)
†Independent variables with >5% missing data
‡ Other careers included homemaker, laborer, service industry worker, or other.

Table 3. Maternal demographics and risk factors for vertical transmission of *T. cruzi* infection

	Women with vertical transmission (n=101) Mean ± SD or n (%)	**Women without vertical transmission (n=1170)** Mean ± SD or n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Demographics				
Age	27.8 ± 7.3	27.5 ± 7.3	1.00 (0.98-1.03)	-
Parity	3.4 ± 1.8	3.2 ± 1.8	1.04 (0.93-1.16)	-
Medical history				
Previous diagnosis of Chagas disease	85 (84.2%)	695 (59.4%)	**3.63 (2.10-6.27)**	-*
Previous treatment for Chagas disease	4	34	1.38 (0.48-3.96)	-*
Family history of Chagas disease	52	694	0.73 (0.48-1.09)	-
History of receiving donated blood	12	184	0.72 (0.39-1.35)	-
Diabetes mellitus	0 (0%)	18	1	-
VDRL † Positive (reference) Negative Not done/unknown	 3 (4.3%) 67 (95.7%) 31	 8 (1.2%) 669 (98.8%) 677	3.74 (0.97-14.5)	-
Toxoplasmosis † Positive (reference) Negative Not done/unknown	 1 (50.0%) 1 (50.0%) 99	 24 (49.0%) 25 (51.0%) 1121	1.04 (0.06-17.6)	-
Socioeconomic factors				
Maternal high school education Yes (reference) No Unknown	 25 (100%) 0 (0%) 0	 371 (31.8%) 797 (68.2%) 2	0.71 (0.44-1.13)	-
Paternal high school education Yes (reference) No Unknown	 36 (100%) 0 (0%) 0	 467 (40.0%) 701 (60.0%) 2	0.83 (0.54-1.27)	-
Maternal career	7 (8.4%)	59 (6.0%)	1.44 (0.63-3.25)	-

Professional or technician (reference) Other ‡ Unknown	76 (91.6%) 18	920 (94.0%) 191		
Paternal career Professional or technician (reference) Other ‡ Unknown	43 (47.3%) 48 (52.7%) 10	390 (38.5%) 624 (61.5%) 156	1.43 (0.93-2.20)	-
Number of people living in household	6.0 ± 2.5	5.6 ± 2.7	1.04 (0.97-1.12)	-
Number of bedrooms in home	2.4 ± 1.8	2.3 ± 1.4	1.08 (0.94-1.23)	-
Number of total rooms in home	4.4 ± 2.1	4.3 ± 1.8	1.03 (0.92-1.15)	-
Number of household amenities	3.0 ± 0.9	3.0 ± 0.9	1.08 (0.86-1.37)	-
Environmental factors				
Having seen triatomine bugs in the home	63 (62.4%)	677 (57.9%)	1.21 (0.79-1.84)	-
Having lived in a home with mud walls	54 (53.5%)	660 (56.4%)	0.89 (0.59-1.33)	-
Having lived in a home with a roof made of palm, straw, or hollow reeds	46 (45.5%)	484 (41.4%)	1.19 (0.79-1.78)	-
Having lived in a home with a dirt floor	50 (49.5%)	623 (53.2%)	0.86 (0.57-1.29)	-
Having lived in a rural area	69 (68.3%)	765 (65.4%)	1.14 (0.74-1.77)	-
Pregnancy and delivery factors				
Cesarean delivery	67 (66.3%)	875 (74.8%)	0.66 (0.43-1.02)	**0.63 (0.41-0.98)**
Premature rupture of membranes	8 (7.9%)	80 (6.8%)	1.17 (0.55-2.50)	-
Twin birth	10 (9.9%)	42 (3.6%)	**2.95 (1.43-6.08)**	**3.17 (1.53-6.55)**

Variables not significantly contributing to the multivariate model were removed from the final reduced model (see Methods).
In the final multivariate model, odds ratios were adjusted for all other variables found to significantly contribute to the model: Cesarean delivery and twin birth.
* Excluded from multivariate model (see Methods)

† Independent variables with >5% missing data
‡ Other careers included homemaker, laborer, service industry worker, or other.

Table 4. Infant demographics and risk factors for congenital Chagas disease in infants born to mothers with Chagas disease

	Infants with congenital Chagas disease (n=113)	Infants without congenital Chagas disease (n=1212)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Being a twin	22 (19.5%)	84 (6.9%)	**3.25 (1.94-5.44)**	**3.30 (1.97-5.54)**
Male sex	67 (59.3%)	604 (49.8%)	1.47 (0.99-2.17)	**1.50 (1.01-2.22)**

Appendix 1. Limited Systematic Review

Introduction

Over 5 million people in Latin America are infected with *Trypanosoma cruzi*, the parasitic agent of Chagas disease.[1] Although transmission most commonly occurs through the triatomine vector ("kissing bug"), vertical transmission from mother to infant accounts for over 20% of new cases.[8] Congenitally infected infants, like other individuals with Chagas disease, have up to a 30% lifetime risk of developing severe and potentially fatal sequelae such as cardiomyopathy, arrhythmias, and gastrointestinal complications.[12]

Antiparasitic therapy is available for *T. cruzi* infection, and congenital Chagas disease is nearly always curable in the first year of life.[13] Likelihood of cure and tolerability of therapy wane with age. However, the majority of infants with congenital Chagas disease do not receive timely diagnosis and treatment. Infant diagnosis is complex, costly, and may take up to a year, as serology cannot be reliably performed until maternal antibody disappears after eight months.[37] Given the limited resources in many endemic setting, better risk stratification is needed to identify maternal, environmental, and sociodemographic characteristics associated with higher rates of vertical transmission.

In this limited systematic review, we aim to identify factors associated with an increased risk of vertical transmission of *T. cruzi* infection. Specifically, we compare characteristics of cases in which women with chronic Chagas disease did transmit the infection to their infants to cases in which they did not. Our review was not limited by publication date, country of origin, or language.

Methods

Search Strategy

Searches were performed for relevant literature in PubMed, LILACS, and Embase electronic databases (Figure A1-1, Table A1-1). A search was also performed in clinicaltrials.gov to identify ongoing or unpublished studies. The last search was performed on March 20, 2020. There was not a registered protocol.

Study Selection

Studies from the aforementioned searches were compiled for further review in Covidence, which removed duplicate studies. One reviewer screened studies by title and abstract using eligibility criteria determined a priori (Table A1-2) and removed irrelevant studies. Studies of mothers with confirmed serology for *T. cruzi* infection and their infants were included. Acceptable exposures included a broad array of potential risk factors for *T. cruzi* transmission, including maternal, parasite, and infant characteristics. Previous maternal treatment for Chagas disease or known infant sequelae of congenital Chagas disease were not considered risk factors. We included studies that compared these exposures among women who did or did not transmit *T. cruzi* infection, or among infants of seropositive women who did or did not develop congenital Chagas disease. We included infants diagnosed with congenital Chagas disease within one year of delivery, as serological diagnosis cannot be performed until after eight months of age, when maternal antibody has disappeared.[37] Infant *T. cruzi* infections diagnosed after one year were excluded as these could represent new acute infections. We included empirical interventional or observational study designs including randomized controlled trials, cohort studies, case control studies, or cross-sectional studies. Review articles, single-patient case studies, letters and comments were excluded. No studies were excluded based on language, date of publication,

publication status, country of origin, or clinical setting. Following title and abstract review, full text articles were screened for inclusion using the same eligibility criteria.

Study Assessment

Risk of bias in individual studies was assessed using the Newcastle-Ottawa Scale (NOS), an accepted quality rating tool for observational studies (Table A1-3).[38] This scale evaluates study quality using a total of eight categories which reflect the selection, comparability, and outcomes of each study. Studies were evaluated by a single reviewer.

Data Extraction

Data extracted for each study included setting, population, study design, Chagas disease prevalence and congenital transmission rate, and risk factors examined (Table A1-4). Study data were extracted manually by a single reviewer. Meta-analysis was not performed given the heterogeneity of study designs and risk factors examined.

Results

Search Strategy

The search identified a total of 549 publications (Figure A1-1). The publications underwent screening by title and abstract, and 493 were determined to be irrelevant. The remaining 56 were screened with full text review. Studies were excluded for ineligible publication type (reviews, single case studies, letters or comments), outcome of interest (did not compare vertical transmission to no vertical transmission), full text unavailable, duplicate, ineligible study design (for example, irrelevant control group), being an ongoing trial with no

study results, ineligible exposure (for example, previous maternal treatment for Chagas disease), or being a poor-quality study (for example, not describing methods for maternal or infant diagnosis of *T. cruzi* infection).

Study Characteristics and Findings

The final analysis included 19 publications, of which two were in Spanish. Study participants were drawn primarily from Bolivia (nine studies) and Argentina (eight studies). Other participant countries included Spain, Mexico, Honduras, and Paraguay.[39–42] The included publications examined a wide variety of potential risk factors for vertical transmission, including maternal age, parasitic load, parasite and infant genetics, having twins, immunologic factors, vector exposure, and more. Individual study designs and findings are summarized in Table A1-4, and key results organized by risk factor are summarized in Table A1-5.

Several studies examined maternal parasitic load as a risk factor for vertical transmission of *T. cruzi* infection. Four studies found a significant association between higher maternal parasitic load and risk of vertical transmission, and no studies reported a non-significant or reduced risk with higher parasitic load.[26,29,34,43] In addition, several studies found an increased risk of vertical transmission among mothers with positive *T. cruzi* PCR, culture, serology, or xenodiagnosis during pregnancy, which may also be suggestive of higher parasitic load.[35,42–46] Maternal vector exposure also appeared to influence risk of vertical transmission. Four studies found that significant historical exposure to triatomine bugs was associated with lower risk of vertical transmission, and two studies found that current home exposure to triatomine bugs was not associated with vertical transmission risk.[29,31,32,34,47]

Other potential risk factors examined by multiple studies included maternal age, having twins, socioeconomic factors, and immunologic factors. In seven studies reporting on risk by maternal age, maternal age was not significantly associated with risk of vertical transmission in six studies.[31,32,34,35,39,43] Only one study found a higher vertical transmission rate among younger women.[21] In three studies assessing risk of vertical transmission among twin births, two found an increased risk of vertical transmission and one found no significant difference.[29,34,35] Two studies found no difference in risk among mothers who lived in urban or rural areas.[31,47] Three studies examined a variety of maternal and infant immunologic factors.[17,45,48] For example, two studies found higher risk of vertical transmission among women with higher IFN-γ levels in cord blood and whole blood cells incubated with *T. cruzi* lysate, respectively.[17,45] Finally, several individual studies considered additional risk factors, such as infant and parasite genetics, maternal HIV status, infant sex, and maternal clinical form of Chagas disease.[21,39–41,46]

Risk of Bias

The risk of bias in the included publications ranged from moderate to high, assessed by the Newcastle-Ottawa Scale (Table A1-3). The most common limitation was inadequate follow-up or response rates, as the majority of infants did not complete the nine-month follow-up required for serological diagnosis. Another common source of potential bias was limited comparability between study groups. The majority of included publications did not control for important confounding factors such as age, medical history, or socioeconomic status.

Discussion

This study is the first systematic review to examine and assess risk factors for vertical transmission of Chagas disease. We identified 19 relevant publications with significant heterogeneity in study design, population, and exposures.

The strongest body of evidence supports an increased risk of vertical transmission among mothers with higher parasitic load. As trypanocidal therapy is contraindicated in pregnant women, this finding suggests that reducing parasitic load in women of childbearing age prior to pregnancy may be a target for reducing vertical transmission. Notably, several studies also found higher transmission rates among women with lower vector exposure.[29,31,32,34] Although somewhat counterintuitive, this relationship is likely explained by differences in parasitic load. Previous studies have found that women who live in infested homes for extended periods actually have lower parasitic loads, leading to the hypothesis that continued re-exposure or superinfection primes the immune system for an enhanced response.[34]

Although older age is a known risk factor for Chagas disease in many endemic countries, six of seven studies that examined maternal age found it was not a significant risk factor for vertical transmission. In addition, some studies suggested that twin births and immunologic factors may play a role in risk of vertical transmission. Maternal immunologic response to *T. cruzi* infection, such as IFN-γ production, may affect susceptibility to vertical transmission; it is unclear whether this susceptibility is mediated by parasitic load. Twin births could also be more susceptible to congenital infection due to placental insufficiency or enhanced immune suppression. More research is warranted to clarify whether twins have an increased risk of vertical transmission, and if so, to study the pathogenesis of this risk.

This limited systematic review has several limitations, including the scope and quality of the existing literature. Common limitations in the studies included lack of comparability between

study groups, lack of control for potential confounding factors, and high loss to follow-up. Poor follow-up is a frequent challenge for congenital Chagas disease studies as infant diagnosis can take up to nine months for serological results.[16,21,36] Many families do not return for long-term follow-up due to financial and travel barriers and low perceived benefit. This study is also limited by the use of a single reviewer to select and evaluate studies.

Conclusions

Despite the high morbidity and mortality associated with congenital Chagas disease, relatively few studies have examined risk factors for vertical transmission of *T. cruzi* infection. Our current understanding of these risk factors is limited by poor to moderate quality evidence and a lack of prospective, randomized trials. However, the existing literature suggests that high maternal parasitic load is associated with elevated risk of vertical transmission of *T. cruzi*, while maternal age does not appear contributory. Additional high-quality research is needed to further elucidate maternal, environmental, infant, and parasitic risk factors for vertical transmission and to identify high-risk populations. As the literature continues to grow, another systematic review is warranted in ten years to reassess risk factors for vertical transmission of *T. cruzi* infection.

Figure A1-1. PRISMA flow diagram

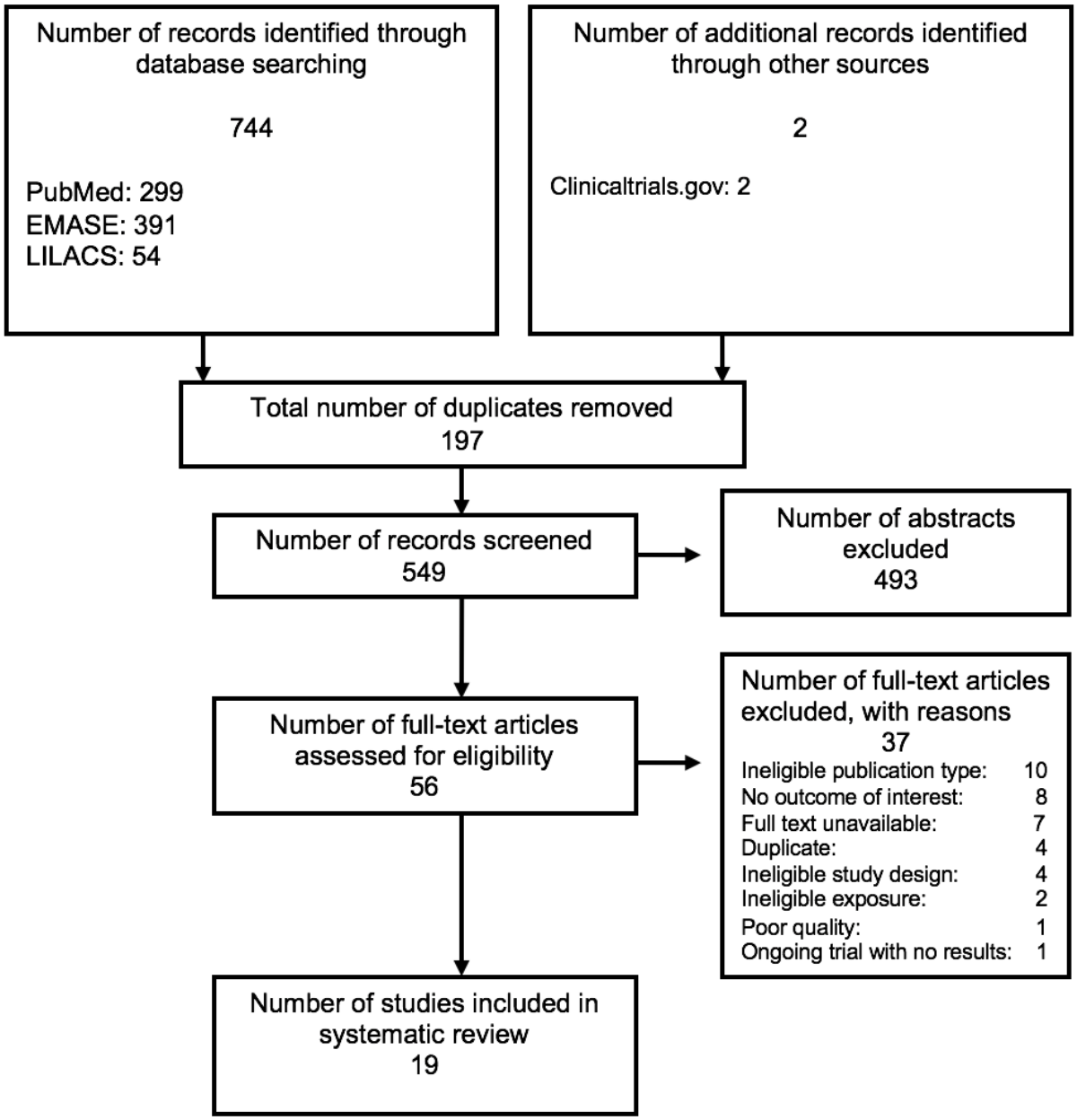


Table A1-1. Search terms

Database	Search Terms	Results (n)	Date Searched
PubMed	("Chagas Disease"[Mesh] OR chagas OR chagas*) AND (vertical* OR congenital*) AND (risk[tw] OR risks[tw] OR risk factors[mesh] OR driver[tw] OR drivers[tw] OR determinant*[tw] OR predict* OR indicat* OR suscep*)	**299**	**3/20/20**
EMBASE	(chagas OR chagasic) AND (vertical OR congenital) AND (risk OR risks OR driver OR drivers OR determinant OR predict OR predictor OR indicate OR indicator OR susceptible OR susceptibility)	**391**	**3/20/20**
LILACS	(chagas OR chagasic) AND (vertical OR congenital) AND (risk OR risks OR driver OR drivers OR determinant OR predict OR predictor OR indicate OR indicator OR susceptible OR susceptibility)	**54**	**3/20/20**
Clinicaltrials.gov	(vertical OR congenital) AND (risk OR risks OR driver OR drivers OR determinant OR predict OR predictor OR indicate OR indicator OR susceptible OR susceptibility) | Chagas Disease	**2**	**3/20/20**

Table A1-2. Inclusion and exclusion criteria

PICOTSS	Inclusion criteria	Exclusion criteria
Population	Mothers with chronic Chagas disease and their infants	Mothers without confirmed serological diagnosis of *T. cruzi* infection
Exposure	Risk factors such as maternal or parasite genetics, parasitic load, living situation, socioeconomic status, maternal nutrition, etc.	Known sequelae of congenital Chagas disease (such as low birth weight or respiratory distress) or previous maternal treatment for Chagas disease
Comparison	Lack of vertical transmission of *T. cruzi* infection from a serologically positive mother to her infant	Other comparisons, such as infants of mothers without chronic Chagas disease
Outcomes	Vertical transmission of *T. cruzi* infection from a serologically positive mother to her infant	
Timing	Diagnosis of congenital Chagas disease within 1 year of delivery	Infant diagnosis after 1 year of age
Setting	Any country, medical setting, publication date, or language	None
Study type	Empirical interventional or observational study designs including randomized controlled trials, cohort studies, case control studies, or cross-sectional studies	Reviews, single-patient case studies, letters or comments

Table A1-3. Risk of bias assessment using Newcastle-Ottawa Scale (NOS).[38] Per NOS guidelines, a maximum of one star can be awarded for each selection or outcome category; a maximum of two stars can be awarded for the comparability category. Overall quality assessment was given as the total number of stars awarded, with a maximum of nine stars.

Study	Selection				Comparability	Outcome			Overall Quality Assessment (Max: 9)
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis (max: 2)	Assessment of outcome	Was follow-up long enough for outcomes to occur?	Adequacy of follow up of cohorts	
Cohort and surveillance studies									
Basile et al., 2019[39]	☆	☆	☆	☆	☆☆	☆	☆		**8**
Bern, 2009[16]	☆	☆	☆	☆		☆	☆		**6**
Brutus et al., 2010[26]		☆	☆	☆		☆	☆		**5**
Kaplinski et al., 2015[29]	☆	☆		☆		☆	☆		**5**
Murcia et al., 2013[42]	☆	☆	☆	☆		☆	☆		**6**
Rendell et al., 2015[34]	☆	☆	☆	☆		☆	☆		**6**
Salas et al., 2007[35]	☆	☆	☆	☆		☆	☆		**6**
Scapellato et al., 2009[46]	☆	☆	☆	☆		☆	☆		**6**
Suasnábar et al., 2018[31]		☆		☆	☆	☆	☆		**5**
Case control studies									
Study	**Selection**				**Comparability**	**Exposure**			**Overall Quality Assessment (Max: 9)**
	Adequate case definition	**Representativeness of cases**	**Selection of controls**	**Definition of controls**	**Comparability of cases and controls (max: 2)**	**Ascertainment of exposure**	**Same method of ascertainment for cases and controls**	**Non-response rate**	
Alonso-Vega et al., 2005[36]	☆		☆	☆	☆	☆	☆		**6**

Burgos et al., 2007[49]	☆		☆	☆		☆	☆		**5**
Chaparro & Genero, 2018[47]	☆		☆	☆	☆		☆		**5**
Hermann et al., 2004[45]	☆		☆	☆		☆	☆		**5**
Herrera et al., 2019[40]			☆	☆			☆		**3**
Juiz et al., 2016[41]	☆		☆	☆	☆	☆	☆		**6**
Messenger et al., 2017[21]	☆	☆	☆	☆		☆	☆	☆	**7**
Sánchez Negrette et al., 2005[32]	☆		☆	☆	☆		☆		**5**
Volta et al., 2016[48]	☆		☆	☆	☆☆	☆	☆		**7**

Table A1-4. Evidence table of included studies.

Study	Setting	Population	Study Design	Chagas Prevalence and Transmission Rates	Risk Factors Evaluated
Alonso-Vega et al., 2005[36]	Cochabamba, Bolivia	Pregnant women and their infants	Case control study	- -	Cord blood cells of seropositive mothers who transmitted the infection had higher levels of IFN-γ and lower levels of IL-10 than those of seropositive mothers who did not transmit (79.4±27.8 vs. 26.1±10.3 pg/mL and 13.7±5.3 vs 5.3±1.6 pg/mL, respectively) (no statistics provided). Mitogen-stimulated cord blood cells of seropositive mothers who transmitted the infection were less likely to produce IL-10 than those of seropositive mothers who did not transmit (35% vs. 7.60%) (no statistics provided). Seropositive mothers who transmitted *T. cruzi* infection were more likely to have a positive culture than seropositive mothers who did not (47% vs. 23%, p<0.05).
Basile et al., 2019[39]	Catalonia, Spain 2010-2015	Pregnant women (n=40,084) and infants	Surveillance study	2.4% seroprevalence in mothers 2.8% transmission rate	Mothers with other children with Chagas disease had higher odds of vertical transmission (OR: 22.79, p=0.001, 95% CI: 3.75-161.54). Mothers with the cardiac form of Chagas disease had higher odds of vertical transmission (OR: 14.40, p=0.009, 95% CI: 2.11-87.67) than mothers with the indeterminate form. Maternal age, previous treatment for Chagas disease, country of birth, and years living in Catalonia were not significantly associated with likelihood of vertical transmission (p=0.693, p=0.093, p=0.801, and p=0.453, respectively).
Bern, 2009[16]	Santa Cruz, Bolivia 2006-2007	Pregnant women (n=530) and their infants	Prospective surveillance study	29% seroprevalence in mothers 6.5% transmission rate	Mothers who transmitted *T. cruzi* infection had higher parasite load than mothers who did not (p<0.01). Mothers with a positive *T. cruzi* PCR result during pregnancy were more likely to transmit the infection (p=0.014). Maternal age and parity were not significantly associated with likelihood of vertical transmission (p=0.64 and p=0.41, respectively).
Brutus et al., 2010[26]	Yacuiba, Bolivia 2004-2005	Pregnant women (n=359) and their infants	Prospective longitudinal study	40.9% seroprevalence in mothers	Mothers with high parasite density had a higher risk of vertical transmission than those with low parasite density (p<0.001).
Burgos et al., 2007[49]	Buenos Aires, Argentina 2000-2006	Infants with congenital Chagas disease (n=47) and their mothers; unrelated seropositive pregnant women who did not transmit the infection (n=32)	Case control study	- -	There was no significant association between *T. cruzi* IId lineage and risk of vertical transmission (p>0.05). Among seropositive mothers, those with positive PCR were more likely to transmit (5/7, 71.4%) than those with negative PCR (13/32, 40.6%) (no statistics provided).

Chaparro & Genero, 2018[47]	Chaco, Argentina 2011	Women with confirmed *T. cruzi* infection (n=247), their infants with suspected infection (n=246), and their siblings (n=556)	Cross sectional study	- 6.1% transmission rate	Newborn health care (taking the infant to a local health center for congenital Chagas disease evaluation) was associated with reduced odds of congenital infection (adjusted OR: 0.21, 95% CI: 0.05-0.82, p=0.02). Knowledge of the infection, rural residence in infancy, living in a home with mud walls, straw roof, or dirt floor, triatomine bugs in the home, blood transfusions, maternal diagnosis with *T. cruzi* infection, annual cardiology care, and household cohabitants were not associated with odds of congenital transmission. Number of siblings was associated with higher odds of congenital infection (adjusted OR: 1.89, 95% CI: 1.43-2.49, p<0.001).
Hermann et al., 2004[45]	Cochabamba, Bolivia Dates not provided	Pregnant women with confirmed *T.* cruzi infection who did (n=24) and did not (n=35) transmit to their infants	Prospective cohort study	- -	More women who transmitted had a positive hemoculture than women who did not transmit (p<0.05). There were no significant differences in secretions of IL-2, IL-4, IL-10, or TGF-β1 between mothers who did or did not transmit. Women who transmitted had higher levels of IFN-γ in whole blood cells incubated with *T. cruzi* lysate than women who did not transmit (p<0.01). Mothers who transmitted had lower proportions of $CD4_+$ HLA-DR_+ T lymphocytes than mothers who did not transmit (p<0.05). Proportions of $CD4_+$ $CD45RO_+$ T cells were similar between groups.
Herrera et al., 2019[40]	Argentina, Honduras, and Mexico	Pregnant women with *T. cruzi* infection (n=100) and their infants	Nested prospective cohort study	- -	*T. cruzi* haplotypes differed between mothers who did and did not vertically transmit the infection (p=0.021).
Juiz et al., 2016[41]	Argentina, Bolivia, and Paraguay	Infants born to *T. cruzi* seropositive mothers (n=217)	Case control study	- 4.7% transmission rate	Infant mutations in the SNPs in the *ADAM12* (rs11244787 and rs1871054) and *MMP2* (rs243866, rs17859821, and rs2285053) genes, which code for placental expression enzymes, were associated with higher odds of transmission. The C>T mutation in rs1871054 was associated with lower odds of transmission (adjusted OR 0.401, 95% CI: 0.227-0.709, p=0.002). The A>G mutation in rs11244787 was associated with higher odds of transmission (adjusted OR 2.085, 95% CI: 1.212-3.589, p=0.008). The A mutation was associated with higher odds of transmission in rs243866 (adjusted OR 49.61, 95% CI: 11.62-211.8, p=0.0001) and rs17859821 (adjusted OR 2.304, 95% CI: 1.301-4.08, p=0.004). The TT allelotype in rs2285053 was associated with higher odds of transmission (adjusted OR 4.903, 95% CI: 1.017-23.65, p=0.048). Other SNPs in *ALPP* (rs2014683 and rs1048988), *MMP2* (rs243865, rs243864, and rs2285053), and *MMP9* (rs3919242 and rs2234681) were not associated with transmission.

Kaplinski et al., 2015[29]	Santa Cruz, Bolivia 2010-2013	Pregnant women (n=1696) and their infants	Prospective cohort study	26.9% seroprevalence in mothers 6.8% transmission rate	Mothers who transmitted *T. cruzi* infection had higher parasite load than mothers who did not ($p<0.0001$). Transmission was higher in twin than singleton births ($p=0.04$). Mothers who had not lived in houses infected with triatomine bugs were more likely to transmit than those that had ($p=0.04$).
Messenger et al., 2017[21]	Santa Cruz, Bolivia 2010-2014	Pregnant women (n=1851) and their infants	Prospective cohort study	25.7% seroprevalence in mothers 7.8% transmission rate	Mothers who transmitted *T. cruzi* infection were younger than mothers who did not ($p<0.01$). Female infants of seropositive mothers were more likely to be diagnosed with congenital Chagas disease than male infants ($p<0.05$).
Murcia et al., 2013[42]	Murcia, Spain 2007-2011	Seropositive pregnant women (n=59) and their infants	Prospective cohort study	- 13.8% transmission rate	Seropositive mothers with a positive *T. cruzi* PCR result during pregnancy were more likely to transmit the infection to their infants than seropositive mothers with a negative PCR results ($p=0.0046$).
Rendell et al., 2015[34]	Santa Cruz, Bolivia 2010-2011	Pregnant women (n=596) and their infants	Prospective cohort study	21.5% seroprevalence in mothers 11.7% transmission rate	Congenital transmission was more common in twin births than singleton births ($p=0.03$). Congenital transmission occurred in 31.3%, 15.4%, and 0% of women with high, moderate, and low parasite loads, respectively (χ_2 for trend 18.2, $p<0.0001$). Women who reported living in an infested house for 1-19 years or 20 or more years had lower odds of congenital transmission than women with 0 years of living in an infected house ($p=0.049$). Living in an infested house or currently living in an infested house were not significantly associated with odds of congenital transmission ($p=0.28$ and $p=0.46$, respectively). Maternal age was not associated with odds of congenital transmission ($p=0.92$).
Salas et al., 2007[35]	Yacuiba, Bolivia 2003-2005	Pregnant women (n=2712) and their infants	Prospective cohort study	42.2% seroprevalence in mothers 5.1% transmission rate	Among all mothers (with and without Chagas disease), positive maternal *T. cruzi* serology was associated with higher odds of transmission (OR: 19.39, $p<0.0001$, 95% CI: 5.95-62.71). Positive maternal parasitemia was also associated with higher odds of transmission (OR: 11.62, $p<0.0001$, 95% CI: 5.92-22.82). Among all mothers (with and without Chagas disease), maternal age, parity, time of residence, being born in Yacuiba, living in a rural area, being without an insecticide house-spraying program, lack of antenatal visit, cesarean section, infant sex, hot season of delivery, and previous stillbirth were not significantly associated with odds of transmission ($p>.05$). Congenital transmission rate was not significantly different between singleton and twin births ($p=0.12$).

Sánchez Negrette et al., 2005[32]	Salta, Argentina 1997-2002	Infants (n=340) born to seropositive mothers	Case control study	- 9.1% transmission rate	Seropositive women from endemic regions with low vector control were less likely to transmit than seropositive women from endemic regions with high vector control (p=0.045). Maternal age, infant sex, and sibling order were not significantly associated with risk of congenital transmission.
Scapellato et al., 2009[46]	Buenos Aires, Argentina 2001-2007	Infants (n=94) born to seropositive mothers	Prospective surveillance study	- 13.8% transmission rate	The transmission rate was higher among children born to HIV+ women (3/3, 100%) than children born to HIV- women (10/91, 10.9%) (difference=0.89, 95% CI: 0.82-0.95, p=0.0021).
Suasnábar et al., 2018[31]	Santa Fe, Argentina 1990-2017	Women (n=83) and infants (n=237) with confirmed *T. cruzi* infections	Retrospective cohort study	- -	Maternal age and history of transfusion were not associated with risk of transmission (p=0.059 and 0.605, respectively). Mothers with medium or high vector exposure had lower risk of transmission than mothers with no or low vector exposure (RR=0.36, 95% CI: 0.14-0.97, p=0.046). Positive xenodiagnosis in seropositive mothers was a risk factor for transmission, controlled for maternal age, transfusion history, and vector exposure (RR=12, 95% CI: 2.9-50.1).
Volta et al., 2016[48]	Buenos Aires, Argentina 2008-2011	Infants diagnosed with congenital Chagas disease diagnosed within 1 month (B^+C1, n=15), 6 months (B^+C2, n=10), or 12 months (B^+C3, n=10); randomly chosen uninfected infants born to seropositive mothers (B^-M^+, n=10); randomly chosen uninfected infants born to seronegative mothers (B^-M^-, n=10)	Retrospective cohort study	-	Congenitally infected infants had higher plasma levels of IL-17A than uninfected infants of seropositive mothers ($p<0.0001$ for all subgroups at 1 month). Congenitally infected infants had higher plasma levels of MCP-1 than uninfected infants of seropositive mothers (p=0.0004 for B^+C1, p=0.0343 for B^+C2, p=0.0274 for B^+C3 at 1 month). Congenitally infected infants had higher plasma levels of monokine induced by IFN-γ than uninfected infants of seropositive mothers ($p<0.0001$ for B^+C1, p=0.0021 for B^+C2, p=0.0232 for B^+C3 at 1 month). Congenitally infected infants had lower plasma levels of IFN-γ than uninfected infants of seropositive mothers ($p<0.0001$ for all subgroups at 1 month). Congenitally infected infants diagnosed after 6 months had higher plasma levels of IL-6 (p=0.0091 for B^+C2, p=0.0184 for B^+C3 at 6 months) and IL-17F (p=0.0261 for B^+C2, p=0.0399 for B^+C3 at 6 months) than uninfected infants of seropositive mothers.

Table A1-5. Summary of evidence by risk factor

**Statistics not provided in the original study for this association*

Risk factor	Studies suggesting increased risk of vertical transmission	Studies suggesting no difference in risk of vertical transmission
Maternal age	One study found an increased risk among younger mothers.[21]	Several studies found no difference in risk with maternal age.[16,30–32,35,39]
Maternal parity	One study found an increased risk in infants with a larger number of siblings.[47]	Two studies found no difference in risk with maternal parity.[16,35]
Parasite load and diagnostics	Several studies found an increased risk with parasite load,[16,26,29,34] positive PCR,[8,16*,17] positive *T. cruzi* serology,[35] parasitemia,[35] positive *T. cruzi* culture[36,45] or positive xenodiagnoses during pregnancy.[31]	
Parasite genetics	One study found apparent clustering of parasite sequences from congenital transmission cases in the TcII-TcV-TcVI cluster.[40]*	One study found no difference in risk with *T. cruzi* IId lineage.[49]
Infant genetics	One study found an increased risk with infant mutations in SNPs of the *ADAM12* (rs11244787, rs1871054) and *MMP2* (rs243866, rs17859821, rs2285053) genes, which code for placental expression enzymes.[41]	One study found no difference in risk with infant mutations in SNPs of the *ALPP* (rs2014683, rs1048988), *MMP2* (rs243865, rs243864, rs2285053), and *MMP9* (rs3919242, rs2234681) genes.[41]
Socioeconomic factors		Two studies found no difference in risk among women who lived in a rural area[35] or lived in a rural area during infancy.[47]
Twins and siblings	Two studies found higher risk in twin than singleton births.[29,30] One study found an increased risk with having other siblings with Chagas.[39]	One study found no difference in risk between twin and singleton births.[35]
Immunologic factors	One study found an increased risk among infants with higher IL-17A, higher MCP-1, higher monokine induced by IFN-γ, and lower IFN-γ.[48] One study found an increased risk among women whose cord blood had higher IFN-γ and lower IL-10.[36]* One study found increased risk among women with higher IFN-γ in whole blood cells incubated with *T. cruzi* lysate and women with lower proportions of $CD4^+$ $HLA\text{-}DR^+$ T lymphocytes.[45]	One study found no difference in risk by maternal IL-2, IL-4, IL-10, or TGF-β1 levels.[45]
Maternal HIV status	One study found an increased risk among women with HIV.[46]	

Vector exposure	Two studies found a higher risk among women who had never lived in a triatomine-infested house than women who had.[29,30] One study found a higher risk among women with low or no vector exposure than women with medium or high vector exposure.[31] One study found a higher risk among women from endemic areas with high vector control than women from endemic areas with low vector control.[32]	Two studies found no difference in risk between women who reported currently having triatomine bugs in the home and women who did not.[30,47]
Infant sex	One study found an increased risk among female infants.[21]	Two studies found that infant sex was not a risk factor.[32,35]
Other	One study found increased risk with the maternal cardiac form of Chagas disease, compared to the indeterminate form.[39]	Two studies found that maternal history of blood transfusion was not a risk factor.[31,47] One study found that sibling order was not a risk factor.[32]